Towards SERS-based on-chip detection of protease activity using nanoplasmonic slot waveguides

N. Turk[1,2], A. Raza[1,2], P. Wuytens[1,2], H. Demol[4,5], A. Skirtach[2,3], K. Gevaert[4,5] and R. Baets[1,2]

[1] Photonics Research Group, INTEC, Ghent University – imec, Belgium
[2] Center for Nano- and Biophotonics, Ghent University, Belgium
[3] Department of Molecular Biotechnology, Ghent University, Belgium
[4] VIB-UGent Center for Medical Biotechnology, Belgium
[5] Department of Biomolecular Medicine, Ghent University, Belgium

Surface Enhanced Raman Spectroscopy (SERS) has already been successfully applied to detect protease activity in a microscope-based experiment. Here, we aimed to translate SERS-based protease activity monitoring to a cheaper, smaller and easier to use waveguide-based on-chip platform. Using nanoplasmonic slot waveguides we have for the first time detected SERS signal of a peptide substrate on a waveguide-based platform and are currently working on demonstrating SERS-based on-chip detection of protease activity.

Introduction

Surface-Enhanced Raman Spectroscopy (SERS) enables selective and sensitive detection of a molecule by acquiring a molecule-specific Raman signal that is enhanced in the proximity of a plasmonic nanostructure. Its specificity and the fact that no labelling is needed, implies that SERS can be efficiently used to detect the activity of proteases. Proteases are enzymes that catalyse the hydrolysis of peptide bonds and which, amongst others, play important roles in various human diseases [1]. They are therefore important drug targets and a real-time, multiplexed method to analyze protease activity is desired for the development of new drugs that inhibit proteases.

Our group previously showed SERS-based detection of protease activity on a model protease (trypsin) in a microscope-based free space measurement using gold nanodomes shown in Figure 1 [2]. Nanodomes are fabricated via nanosphere lithography, a method that provides a large quantity of chips while minimizing cost and effort. It additionally avoids e-beam lithography, but still offers good control of the gap size and, consequently, the SERS enhancement compared to colloidal approaches.

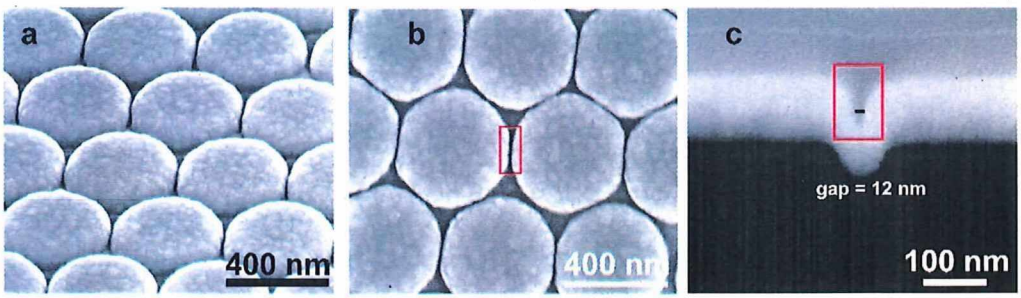

Figure 1: Gold nanodomes with an average gap size of about 12 nm. **a** 52° tilted view. **b** Top-down view. **c** Cross-section of a nanodome gap.

As shown in Figure 2, we label the nanodomes with a substrate for trypsin, i.e. a peptide also containing two aromatic amino acids that provide two non-overlapping SERS peaks.

After trypsin cleaves this peptide, we observe a decrease of the SERS peak corresponding to the cleaved-off part of the peptide. Thus, the SERS peak intensity ratio provides a metric for protease activity.

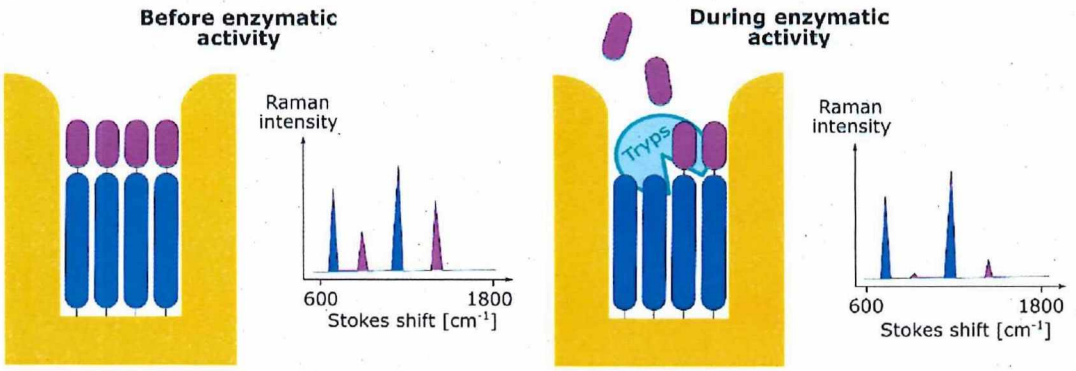

Figure 2: SERS-based detection of trypsin activity.

Towards multiplexing with non-natural aromatic amino acids

Our approach also allows multiplexing measurements in which we monitor the activity of two (or more) different proteases by using protease-specific peptide substrates with non-overlapping SERS peaks (Figure 3). Since there are only three natural aromatic amino acids providing SERS signals (phenylalanine, tryptophan and tyrosine), we needed to turn to non-natural aromatics to allow for multiplexing.

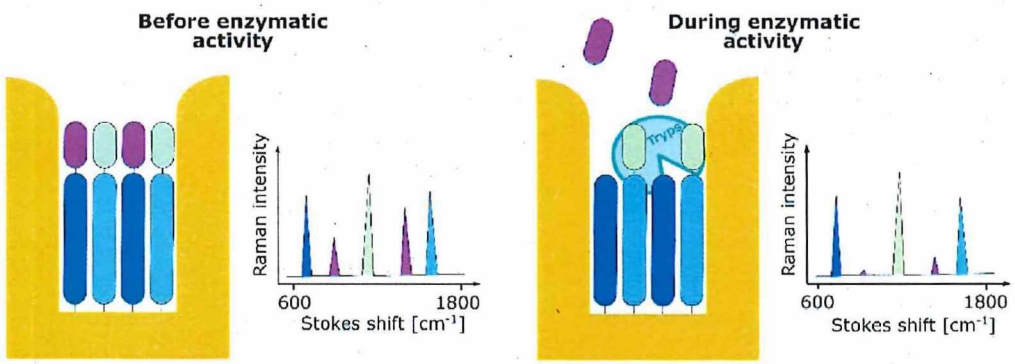

Figure 3: Multiplexing experiment where we detect the activity of two proteases.

As a first step towards multiplexed measurements, we have successfully incorporated the non-natural aromatic amino acid cyano-phenylalanine (CN-F) into a peptide substrate for trypsin (Figure 4). The peptide starts with a cysteine residue (C) that anchors the peptide to the surface of the gold nanostructure via a gold-sulphur bond [3]. The sequence CALNN is required to create a peptide monolayer on the gold surface [4], followed by two aromatic cyano-phenylalanines (CN-F) separated by a serine (S). Two cyano-phenylalanines provide increased SERS signals however, as these are hydrophobic, we separated them with a hydrophilic serine to ensure peptide solubility in aqueous buffers. Next, a series of glycines (GGGG) ensures accessibility of the cleavage site for the protease. The sequence VR serves as the specific cleavage site for trypsin [5], after which the two phenylalanines separated by serine (FSF) provide the second SERS peak. Hence,

the ratio of the peak intensities of F/CN-F is a metric for tryptic cleavage and subsequent diffusion of the -GNFSF fraction.

Figure 4: Peptide substrate for trypsin, with single-letter amino acid code. White letters represent aromatic amino acids that provide SERS signals, namely phenylalanine (F) and the non-natural cyano-phenylalanine (CN-F).

Using nanodomes we demonstrated that trypsin efficiently cleaved this peptide, resulting in an approximate 30% decrease in the intensity of the SERS peak of phenylalanine (F) at 1003 cm^{-1} as shown in Figure 5a. In our experiments, we used nanodomes with gap sizes of about 12 nm, which are small enough to provide high SERS enhancement factor, but still accessible to trypsin that has an effective radius of 2-3 nm. However, small gap sizes limit the accessibility of the peptide substrate, which means that trypsin is not able to cleave all the peptide substrates in these gaps and we were therefore not able to observe complete disappearance of the F peak. In Figure 5b we show that the decrease in the F peak is indeed caused by trypsin cleavage of the peptide substrate, as no change in the SERS spectra is detected in the presence of inactivated trypsin or when no trypsin is added.

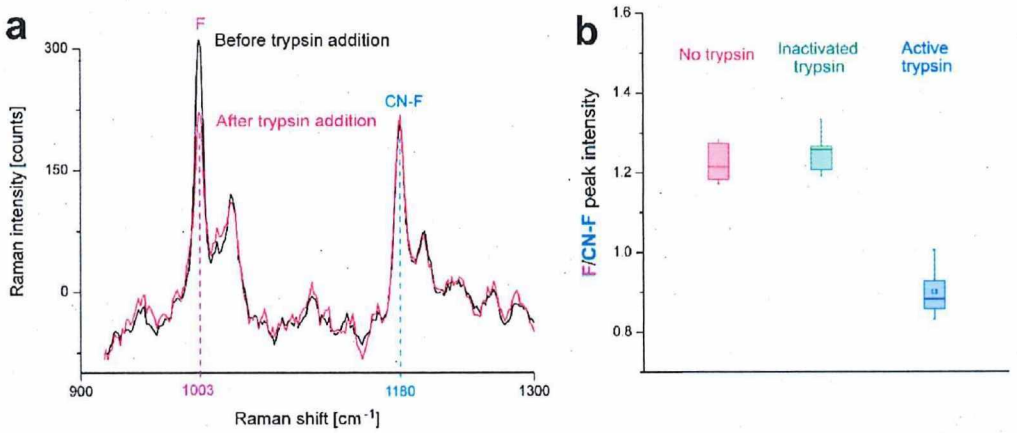

Figure 5: **a** SERS spectra before and after trypsin addition. Addition of trypsin results in approximately 30% decrease in the intensity of the SERS peak of phenylalanine (F) at 1003 cm^{-1}. **b** The F/CN-F peak intensity ratio only decreases after trypsin addition.

Nanophotonic slot waveguides for on-chip detection of protease activity

To avoid the use of bulky and expensive Raman microscopes, we intend to translate our SERS-based protease activity measurements to a cheaper, smaller and easier to use waveguide-based on-chip platform. Nanoplasmonic slot waveguides (Figure 6a) provide high SERS enhancements while avoiding electron beam lithography, making the fabrication of these structures compatible with large-scale production [6]. To fabricate nanophotonic slot waveguides, silicon nitride (Si_3N_4) waveguides are first fabricated using deep UV lithography, followed by a deposition of a uniform layer of aluminum oxide (Al_2O_3) using atomic layer deposition. Finally, a gold layer (Au) is deposited to create a plasmonic gap of approximately 15 nm.

Using doubled aromatic amino acids in the peptide substrate, we increased its SERS signal. Combining such an improved peptide substrate with nanoplasmonic slot waveguides with high SERS enhancements allowed us to detect SERS signals of a peptide substrate on a waveguide-based platform for the first time (Figure 6b). We are currently working on improving the stability of the nanophotonic slot waveguide SERS signal, which should allow us the first demonstration of SERS-based on-chip detection of protease activity.

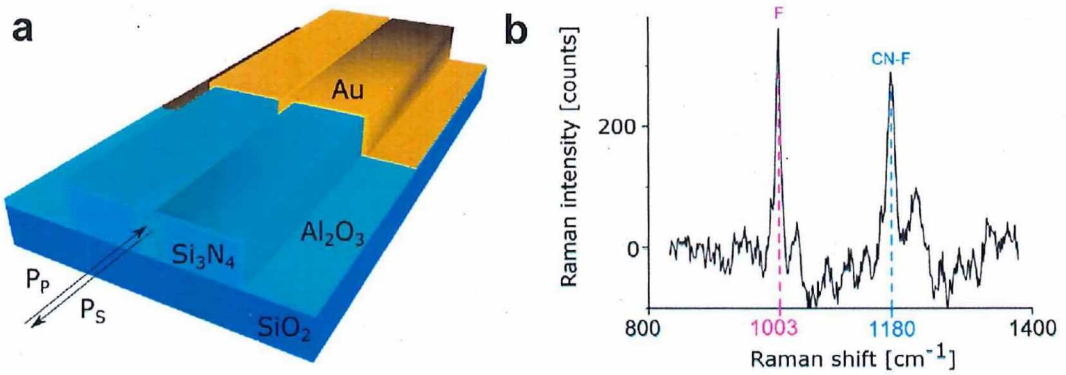

Figure 6: **a** Nanoplasmonic slot waveguides provide high SERS enhancement while avoiding the use of electron beam lithography [6]. **b** SERS signal of a peptide on a waveguide-based platform, detected for the first time.

Conclusions

Following proof-of-concept detection of protease activity on a gold nanodome platform [2], we intend to expand our platform to multiplexed measurements. To achieve this, we used a combination of natural and non-natural aromatic acids that provide non-overlapping SERS peaks. We showed that our model protease trypsin efficiently cleaved a peptide substrate harboring the non-natural aromatic cyano-phenylalanine. In parallel, we have used the same peptide substrate to demonstrate for the first time the detection of SERS signals of a peptide on a waveguide-based platform using nanoplasmonic slot waveguides. We are currently working towards the proof-of-concept demonstration of SERS-based on-chip detection of protease activity.

References

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